

In re Application of:
Sukumar, *at al.*
Application No.: 10/059,579
Filed: January 28, 2002
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PATENT
ATTY. DOCKET NO.: JHU1630-1

AMENDMENT

In the Specification:

Following the abstract, please insert the attached Sequence Listing with subsequent page numbering thereafter.

Please amend the specification as follows:

Please enter the following replacement paragraphs:

[0016] **Figure 1A** shows the nucleotide sequence of the cyclin D2 promoter (SEQ ID NO:105). Regions highlighted indicate primer sequences. CG nucleotide pairs are shown capitalized and bolded. A highlighted box shows the location of an atg codon. **Figure 1B** shows nucleotide sequences for forward (F) and reverse (R) primer external and internal pairs (SEQ ID NOS 21 - 24, 129 and 130) used to detect methylated (M) and unmethylated (U) nucleic acids. The base pair (BP) length of the primer pair product is also indicated.

[0017] **Figures 2A and 2B** show the nucleotide sequence of the TWIST promoter (SEQ ID NO:106). Regions highlighted indicate primer sequences. CG nucleotide pairs are shown capitalized and bolded. A highlighted box shows the location of an atg codon. **Figure 2C** shows nucleotide sequences for forward (F) and reverse (R) external and internal primer pairs (SEQ ID NOS 109, 110, 107, 108, 131 and 132) used to detect methylated (M) and unmethylated (U) nucleic acids. The base pair (BP) length of the primer pair product is also indicated.

[0018] **Figure 3A** shows the nucleotide sequence of the Retinoic Acid Receptor Beta (RAR β) promoter (SEQ ID NO:91). Regions highlighted indicate primer sequences. CG nucleotide pairs are shown capitalized and bolded. A highlighted box shows the location of an atg codon. **Figure 3B** shows nucleotide sequences for forward (F) and reverse (R) external and internal primer pairs (SEQ ID NOS 92, 93, 135, 136, 133 and 134) used to detect methylated

(M) and unmethylated (U) nucleic acids. The base pair (BP) length of the primer pair product is also indicated.

[0019] **Figure 4A** shows the nucleotide sequence of *Homo sapiens* serine protease-like protease (NES-1) mRNA (SEQ ID NO:94). **Figure 4B** shows the nucleotide sequence of the NES-1 region (exon 3) analyzed (SEQ ID NO:95). Regions highlighted indicate primer sequences. CG nucleotide pairs are shown capitalized and bolded. **Figure 4C** shows nucleotide sequences for forward (F) and reverse (R) primer pairs (SEQ ID NOS 77 – 80) used to detect methylated (M) and unmethylated (U) nucleic acids. The base pair (BP) length of the primer pair product is also indicated.

[0020] **Figure 5A** shows the nucleotide sequence of HOXA5 promoter (SEQ ID NO:96). CG nucleotide pairs are shown capitalized and bolded. A highlighted box shows the location of a cat codon. **Figure 5B** shows the nucleotide sequence of the complementary region (5' to 3'') analyzed (nucleotides -97 to -303) (SEQ ID NO:97). Regions highlighted indicate primer sequences. CG nucleotide pairs are shown capitalized and bolded. Highlighted box shows an atg codon. **Figure 5C** shows nucleotide sequences for forward (F) and reverse (R) primer pairs (SEQ ID NOS 71, 98, 72, 69, 99 and 70) used to detect methylated (M) and unmethylated (U) nucleic acids. The base pair (BP) length of the primer pair product is also indicated. **Figure 5D** shows forward and reverse (sense and antisense) primers (SEQ ID NOS 73, 100, 74, 75, 101, 76) used for sequencing and expression of HOXA5.

[0023] **Figure 8A and 8B** show the nucleotide sequence of *Homo. sapiens* (SEQ ID NO:104) estrogen receptor beta gene, promoter region and partial cds. CG nucleotide pairs are shown capitalized and bolded. **Figure 8C** shows nucleotide sequences of forward (F) and reverse (R) primer (SEQ ID NOS 85, 86, 87, 88, 89 and 90) pairs used to detect methylated (M) and unmethylated (UM) nucleic acids. The base pair (BP) length of the primer pair product is also indicated.

[0024] **Figure 9A** shows the nucleotide sequence of human HIN-1 (SEQ ID NO:120) cDNA Regions highlighted indicate primer sequences. **Figure 9B** shows nucleotide sequences of forward and reverse external and internal primer pairs used to detect methylated and unmethylated nucleic acids. The base pair (bp) length of the primer pair product is also indicated.

[0025] **Figure 10A** shows the nucleotide sequence of the RASSF1A promoter (SEQ ID NO:121). CG nucleotide pairs are shown capitalized and bolded. Regions highlighted indicate primer sequences. **Figure 10B** shows nucleotide sequences of forward (F) and reverse (R) external and internal primer pairs (SEQ ID NOS 122, 123, 124, 126, 127 and 128) used to detect methylated (M) and unmethylated (UM) nucleic acids. The base pair (BP) length of the primer pair product is also indicated.

[0033] The nucleic acid of interest can be any nucleic acid where it is desirable to detect the presence of a differentially methylated CpG island. The CpG island is a CpG rich region of a nucleic acid sequence. The nucleic acids includes, for example, a sequence encoding the following genes (GenBank Accession Numbers are shown, followed by the nucleotides corresponding to the region(s) examined for the presence or absence of methylation (numbers are relative to the first ATG codon unless otherwise indicated)): Twist (Accession No. AC003986; -51145 to 151750 (complement) (SEQ ID NO:106) , cyclin D2 (Accession No. U47284; -1616 to -1394) (SEQ ID NO:105); RAR β 2 (Accession No. AF; 157484; -196 to -357)(SEQ ID NO:91), WT1 (Accession No. AB034940) (SEQ ID NO:103); HOXA5 (Accession No. AC004080) (SEQ ID NO:96), 14.3.3 sigma (Accession No. AF029081) (SEQ ID NO:102); estrogen receptor (ER; Accession No. X62462) (SEQ ID NO:104); NES-1 (Accession No. AF024605) (SEQ ID NO:94), the nucleotide sequence of each of which is incorporated by reference herein.

TABLE 4

SEQ ID NO:	Gene	Sense/ antisense		
1	WT	Sense	5'-GCGGCGCAGTTCCCCAACCA-3'	nucleotides 882-901
2	WT	antisense	5'-ATGGTTTCTCACCAGTGTGCTT-3'	nucleotides 1416-1437
3	WT	Sense	5'-GCATCTGAAACCAGTGAGAA-3'	nucleotides 1320-1339
4	WT	antisense	5'-TTTCTCTGATGCATGTTG-3'	nucleotides 1685-1702
5	WT	Sense	5'-GATTGGCTACCCAACTGTTGCA-3'	
6	WT	antisense	5'-CAGGGGCAGCAGCCACAAAGGC-3'	
7	WT	sense	5'-TTTGGGTTAAGTTAGGCGTCGTCG-3'	
8	WT	antisense	5'-ACACTACTCCTCGTACGACTCCG-3'	
9	WT	sense	5'-TTTGGGTTAAGTTAGGTGTTGTTG-3'	
10	WT	antisense	5'-ACACTACTCCTCATACTCACTCCA-3'	
11	WT	sense	5'-CGTCGGGTGAAGGCGGGTAAT-3'	
12	WT	antisense	5'-CGAACCCGAACCTACGAAACC-3'	
13	WT	sense	5'-TGTTGGGTGAAGGTGGGTAAT-3'	
14	WT	antisense	5'-CAAACCCAAACCTACAAAACC-3'	
15	cyclin D2	sense	5'-CATGGAGCTGCTGTGCCACG -3'	
16	cyclin D2	antisense	5'-CCGACCTACCTCCAGCATCC -3'	
17	cyclin D1	sense	5'-AGCCATGGAACACCAGCTC-3'	
18	cyclin D1	antisense	5'-GCACCTCCAGCATCCAGGT-3'	
19	cyclin D2	sense	5'-GATTGGCTAC CCAACTGTTGCA-3'	
20	cyclin D2	antisense	5'-CAGGGGCAGCAGCCACAAAGGC-3'	
21	cyclin D2	sense	5'-GTTATGTTATGTTTGTGTATG-3'	unmethylated
22	cyclin D2	antisense	5'-GTTATGTTATGTTTGTGTATG-3'	unmethylated
23	cyclin D2	sense	5'-TACGTGTTAGGGTCGATCG-3'	methylated
24	cyclin D2	antisense	5'-CGAAATATCTACGCTAAACG-3'	methylated
129	cyclin D2	sense	5'-TATTTTTTTGTAAGATAGTTTTGAT-3'	External
130	cyclin D2	antisense	5-TACAACCTTTCTAAAAATAACCC-3'	External
25	14.3.3 sigma	sense	5'-ACAGGGGAACCTTATTGAGAGG-3'	A 375 bp σ -specific probe
26	14.3.3 sigma	antisense	5'-AAGGGCTCCGTGGAGAGGG-3'	(SEQ ID NO:26)
27	14.3.3 sigma	sense	5'-GAGGAGTGTCCTGCTTGTGG-3'	A TG repeat sequence in the 3'UTR of σ
28	14.3.3 sigma	antisense	5'-GTCTCGGTCTTGCACTGGC3'	

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SEQ ID NO:	Gene	Sense/ antisense		
29	14.3.3 sigma	sense	5'-GTGTGTCCCCAGAGCCATGG-3'	A 1.2 kb PCR product, encompassing the entire σ coding sequence, was generated using two primers
30	14.3.3 sigma	antisense	5'-GTCTCGGTCTTGCACTGGCG-3'	(antisense; SEQ ID NO:30)
31	14.3.3 sigma	antisense	5'-CACCTTCTCCCGGTACTCACG-3'	entire σ coding sequence:
32	14.3.3 sigma	sense	5'-GAGCTCTCCTGCGAAGAG-3'	entire σ coding sequence:
33	14.3.3 sigma	sense	5'-GAGGAGGCCATCCTC TCTGGC-3'	entire σ coding sequence:
34	14.3.3 sigma	antisense	5'-TCCACAGTGTGAGGTTGTCTCG-3'	entire σ coding sequence:
35	14.3.3 sigma, first exon	sense	5'-GAGAGAGTTAGTTTGAATTTAGAAG-3'	start at nt 8641 generates a 474 bp PCR product
36	14.3.3 sigma	antisense	5'-CTT ACTAATATCCATAACCTCC-3'	(antisense primer with start at nt 9114;
37	14.3.3 sigma	sense	5'-TGGTAGTTTTTATGAAAGGCGTC-3'	methylated DNA
38	14.3.3 sigma	antisense	5'-CCTCTAACCGCCACACG-3'	
39	14.3.3 sigma	sense	5'-ATGGTAGTTTTTATGAAAGGTGTT-3'	unmethylated DNA
40	14.3.3 sigma	antisense	5'-CCCTCTAACCACCCACCACA-3'	
41	14.3.3 sigma	sense	5'-GTGTGTCCCCAGAGCCATGG-3'	PCR was performed using the σ -specific primers

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SEQ ID NO:	Gene	Sense/antisense		
42	14.3.3 sigma	antisense	5'-ACCTTCTCCCGGTACTCACG-3'	
43	RAR β	sense	5'-AGA GTT TGA TGG AGTTGG GTG GAG-3'	227 bp probe was amplified
44	RAR β	antisense	5'-CAT TCG GTT TGGGTC AAT CCA CTG-3'	
45	RAR β	sense	5'-CAGCCCGGGTAGGGTTCACC-3'	W3
46	RAR β	antisense	5'-CCGGATCCTACCCCGACGG-3'	W3
47	RAR β	sense	5'-CCGAGAACGCGAGCGATCC-3'	W4
48	RAR β	anti-sense	5'-GGCCAATCCAGCCGGGGCG-3'	W4
49	RAR β	sense	5'-GTG GGT GTA GGT GGA ATA TT-3'	unmethylated DNA were as follows: U1
50	RAR β	antisense	5'-AAC AAA CAC ACA AAC CAA CA-3'	U1
51	RAR β	sense	5'-TGT GAG TTA GGA GTA GTG TTTT-3'	U2
52	RAR β	antisense	5'-TTC AAT AAA CCC TAC CCA-3'	U2
53	RAR β	sense	5'-TTA GTA GTT TGG GTA GGGTTT ATT-3'	U3
54	RAR β	antisense	5'-CCA AAT CCT ACC CCAACA-3'	U3
55	RAR β	sense	5'-GAT GTT GAG AAT GTGAGT GAT TT-3'	U4
56	RAR β	antisense	5'-AAC CAA TCC AACCAA AAC A-3'	U4
57	RAR β	sense	5'-AGC GGGCGT AGG CGG AAT ATC-3'	methylated M1
58	RAR β	antisense	5'-CAACGA ACG CAC AAA CCG ACG-3'	M1
59	RAR β RAR β	sense	5'-CGT GAG TTA GGA GTA GCG TTT C-3'	M2
60	RAR β	antisense	5'-CTT TCG ATA AAC CCT ACC CG-3'	M2
61	RAR β	sense	5'-GGT TAG TAG TTC GGG TAG GGTTTA TC-3'	M3
62	RAR β	antisense	5'-CCG AAT CCT ACC CCGACG-3'	M3
63	RAR β	sense	5'-GTC GAG AAC GCG AGCGAT TC-3'	M4
64	RAR β	antisense	5'-CGA CCA ATC CAA CCGAAA CG-3'	M4
65	RAR β	sense	5'-GAC TGT ATG GAT GTTCTG TCA G-3'	RT \pm PCR exon 5
66	RAR β	antisense	5'-ATT TGTCCT GGC AGA CGA AGC A-3'	exon 6
133	RAR β	sense	5'-GTAGGAGGGTTTATTT TTTGTT-3'	External
134	RAR β	antisense	5'-AATTACATTTTCCAAACTTACTC-3'	External
135	RAR β	sense	5'-GA ACGCGAGCGATTTCGAGT-3'	Methylated
136	RAR β	antisense	5'-GACCAATCCAACCGAAACG-3'	Methylated
92	RAR β	sense	5'-GGATTGGGATGTTGAGAATGT-3'	Unmethylated
93	RAR β	antisense	5'-CAACCAATCCAACCAAAACAA-3'	Unmethylated
67	Actin	sense	5'-ACC ATG GAT GAT GAT ATCG-3'	RT \pm PCR
68	Actin	antisense	5'-ACA TGG CTG GGG TGTGA AG-3'	
69	HoxA5	sense	5'-TTTAGCGGTGGCGTTCG-3'	methylated DNA
70	HoxA5	antisense	5'-ATACGACTTCGAATCACGTA-3'	
71	HoxA5	sense	5'-TTGGTGAAGTTGGGTG-3'	unmethylated

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SEQ ID NO:	Gene	Sense/ antisense		
72	HOXA5	antisense	5'-AATACAACCTCAAATCACATAC-3'	
73	HOXA5	sense	5'-ATTTTGTATAATGGGTTGTAAT3'	
74	HOXA5	antisense	5'-AACATATACTTAATTCCTCC-3'	
75	HOXA5	sense	5'-TCATTTTGC GGTCGCTATCC-3'	RT-PCR
76	HOXA5	antisense	5'-GCCGGCTGGCTGTACCTG-3'	
77	NES-1	sense	5'-TTGTAGAGGTGGTGTGTTT-3'	unmethylated
78	NES-1	antisense	5'-CACACAATAAAACAAAAACCA -3'	
79	NES-1	sense	5'-TTCGAAGTTTATGGCGTTTC-3'	Methylated
80	NES-1	antisense	5'-TTATTTCCGCAATACGCGAC-3'	
81	NES-1	sense	5'-ACCAGAGTTGGGTGCTGAC-3'	
82	NES-1	antisense	5'-ACCTGGCACTGGTCTCCG-3'	
83	36B4	sense	5'-GATTGGCTACCCAACTGTTGCA-3'	
84	36B4	antisense	5'-CAGGGGCAGCAGCCACAAAGGC-3'	
85	Estrogen Receptor	sense	5' -G GGTGTTTTT AGATTGTTGG- 3	Unmethylated
86			5'-TG AGTTGTGATG GGTTTTGG-3	
87		antisense	5'-CCAAAACC CATCACAAC CA-3	
88		sense	5'-AGAGTAGGCG GCGAGCGT-3	Methylated
89			5'-CGGGAAAAG TACGTGTTTCG T-3	
90		antisense	5'-A CGAACACGTA CTTTCCCG-3	
107	Twist	sense	5' -T TTCGGATGGG GTTGTATC- 3	Methylated
108	Twist	antisense	5' -AAACGAC CTAACCCGAA CG- 3	Methylated
109	Twist	sense	5' -TT TGGATGGGGT TGTATTGT- 3	Unmethylated
110	Twist	antisense	5' -C CTAACCCAAA CAACCAACC- 3	Unmethylated
131	Twist	sense	5' -GAGATGAGATATTATTATTGTG- 3	External
132	Twist	antisense	5' -AACAACAATATCATTAAACCTAAC- 3	External
111	HIN-1	sense	5'-AGGGAAGTTTTTTTATTGGTT-3	
112	HIN-1	antisense	5'-GTGGTTTTGTTTTGTATGTTTTGGTG-3	
113	HIN-1	antisense	5'-CACCGAAACATACAAAACAAACCAC-3	
114	HIN-1	sense	5'-GTTTGTTAAGAGGAAGTTTT-3	External
115	HIN-1	antisense	5'-CACCGAAACATACAAAACAAACCAC-3	External
116	HIN-1	sense	5'-GGTACGGGTTTTTTACGGTTCGTC-3	Methylated
117	HIN-1	antisense	5'-AACTTCTTATACCCGATCCTCG-3	Methylated
118	HIN-1	sense	5'-GGTATGGGTTTTTTATGGTTTGT-3	Unmethylated
119	HIN-1	antisense	5'-CAAAACTTCTTATACCCAATCCTCA-3	Unmethylated
122	RASSF1A	sense	5'-GGGAGTTTGAGTTTATTGAGT-3	External
123	RASSF1A	antisense	5'-ACCCCTTAACCTACCCCTTC-3	External
124	RASSF1A	sense	5'-GTTGGTATTC-3	Methylated
125	RASSF1A	sense	5'-GTTGGGCGC-3	Methylated
126	RASSF1A	antisense	5'-GCACCACGTATACGTAACG-3	Methylated
127	RASSF1A	sense	5'-GGTGTATTTGGTTGGAGTG-3	Unmethylated
128	RASSF1A	antisense	5'-CTACAAACCTTACACACAACA-3	Unmethylated